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Song as a signal to negotiate a sexual conflict?

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Abstract—Sexual conflict occurs when the optimal solution regarding e.g. a life history trait differs between co-operating individuals of different sex. When deciding a conflict is not instantaneous, some form of negotiation can be expected to evolve. In great tits, *Parus major*, a sexual conflict exists over the number of clutches that are reared, because the fitness costs of a second clutch are greater for females. A conflict is also likely to exist over investment in the first brood — each parent benefiting from a greater investment by the partner. Male great tits sing when rearing the first brood, and if acoustic signals play a role in the negotiation of a sexual conflict, a positive association between male song rate and maternal investment is predicted. In agreement with this hypothesis, maternal effort (in kJ/day) relative to paternal effort was positively correlated with male song rate. Furthermore, females were more likely to start a second clutch when their male had a high song rate, and high song rate was associated with shorter inter-clutch intervals. Song rate was higher when brood size was experimentally reduced and, independent of brood size manipulation, males with high song rate produced higher quality fledglings. These results indicate that song rate reflects the males' state, suggesting it may function as a handicap signal. Although song rate seems too low (<4% of time) for honesty to be maintained by production costs alone, signalling costs may be amplified by the fact that song appears restricted to the time when the male and female are both near the nest. To achieve a high song rate, the male may have to spend a large amount of time near the nest, thereby seriously restricting time available for other activities.

Keywords: daily energy expenditure; differential allocation; multiple breeding; *Parus major*.

INTRODUCTION

Experiments in which sexual conflict was reduced have elegantly demonstrated the fitness costs of sexual conflict (Holland and Rice, 1999; Royle et al., 2002), indicating that sexual conflict and the ensuing arms race can exert strong selection on sexual behaviour. Given that a conflict exists, some form of negotiation can be expected to evolve to decide the conflict (McNamara et al., 1999), at least when

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decisions are not instantaneous. The subject of this paper is the hypothesis that song rate plays a role in the negotiation of sexual conflicts in the great tit *Parus major*.

In birds, sexual conflict has been demonstrated over diverse aspects of reproduction such as fertilisation, hatching asynchrony and provisioning rate (review, Lessells, 1998). Great tits are facultative multiple breeders: a variable proportion of the population starts a second clutch after successfully fledging young from the first clutch (Verboven and Verhulst, 1996). Experiments in which the second clutch was removed demonstrated that there are fitness costs attached to rearing a second clutch, and females suffered significantly higher costs than males (Verhulst, 1998). Thus there will be conditions where a second clutch is beneficial only to males, and hence there will be a sexual conflict over the decision to start a second clutch.

The reproductive state and behaviour of females can be directly affected by male vocalisations. For example, playback of vocalisations alone stimulated nest building and egg laying in budgerigars and canaries (Brockway, 1965; Hinde and Steel, 1976). Great tit males sing when rearing the first brood (van Duyse et al., 2000), and this could therefore be part of the negotiation over parental effort and the decision to start a second clutch. A direct test of this hypothesis would involve large-scale playback experiments (Logan et al., 1990), but this falls outside the scope of the present study. Instead I evaluate in free-living great tits how song rate correlates with the division of labour and multiple breeding, and how song rate is associated with two factors (hatch date, brood size) that have been shown experimentally to affect multiple breeding (Smith et al., 1987; Tinbergen, 1987; Verboven and Verhulst, 1996).

METHODS

The data reported in this paper were collected in 1990 and 1991 on Vlieland, an island in the Dutch Wadden Sea. There is one large woodland on Vlieland (the village wood), and four smaller woodlands. The woodlands consist mainly of pine, with patches of oak (see Verhulst and van Eck, 1996, for more information). Nest boxes were checked at least once a week during the breeding season. Clutch size was recorded, and laying date was calculated assuming a laying rate of one egg per day. Nests were checked daily around the expected day of hatching to establish the hatching date. Adults were captured with spring traps when the young were 7-14 days old, and subsequently identified using numbered metal rings. When the young were 15 days old (day of hatching = 0) they were ringed, and mass, tarsus and wing length were measured.

Clutch size of the first clutch of a pair was either reduced or unmanipulated; there were no clutch enlargements (see Verhulst and Tinbergen, 1997, for details of the experimental procedure). In brief, dyads of clutches with the same laying date and clutch size were selected and in one randomly chosen clutch in each dyad 50% of the eggs were replaced by artificial eggs. The artificial eggs were removed from the nest 1 day after the first eggs hatched.

Experiments were carried out in all woodlands, but behavioural observations and measurements of parental energy expenditure were restricted to the village wood. Nest boxes were observed with a telescope to record parental behaviour from as great a distance as vegetation permitted (usually 50-100 m). Observations started between 07:20 and 16:30 C.E.T. (95% interval), and thus did not include the dawn chorus. All observations were of pairs with chicks (mean chick age = 9.8, s.d. = 3.0, n = 53), and, with a few exceptions, each observation period lasted 2 h. There was not more than one observation period per nest box per day. Each visit to the nest box, and the time of visit, was recorded for both sexes. Time spent singing (ignoring other vocalisations such as alarm calls) was recorded using a stopwatch. This was facilitated by the low density of breeding pairs (Verhulst and van Eck, 1996), and by the fact that males usually sang in the vicinity of their nest box. Observations used in this paper were during the nestling phase of 'early clutches' only, excluding replacement clutches and second clutches (see Verhulst and Tinbergen, 1997, for methods used to measure energy expenditure). Observations done to verify whether birds had resumed work after having been injected with doubly labelled water were omitted.

Data were analysed with general linear models with normal or binomial error distribution, and partial correlations. Song rate was arcsine square root transformed prior to analyses.

RESULTS

In total we recorded the song rate of 53 males, on average 6.2 h (s.d. = 3.9) per male, spread over 3.2 (s.d. = 2.0) observation periods. To avoid pseudo-replication, song rate was summarised per male, after investigating possible effects of hatch date, age of young and time of day in a multiple regression model. Controlling for year, song rate was independent of time of day, and time of day squared (both $P > 0.8$). Song rate declined with age of the young ($P < 0.001$), but interactions of nestling age with year, hatch date, multiple breeding, and brood size manipulation were all non-significant (all $P > 0.3$). Furthermore, the effect of age was linear, because there was no significant effect of age squared ($P > 0.3$). Therefore the analysis of the effect of brood size manipulation and other factors on song rate was done on average song rate per male, and the effect of nestling age was controlled for by including mean age at the observations in the analyses. Observation date and nestling age did not differ between control and experimental broods (both $P > 0.6$), but observations were on average at younger age in the second year (2.6 days, $P < 0.01$). Hatch date was 4 days later in the second year ($P < 0.01$).

To verify that we could measure individual variation in song rate I calculated the repeatability of our measurements (following Becker, 1984; Lessells and Boag, 1987), comparing measurements of song rate on different days of rearing a particular brood (n = 37 broods with more than one observation period). Repeatability of song rate corrected for nestling age was reasonable ($r = 0.37$, s.e. = 0.09,

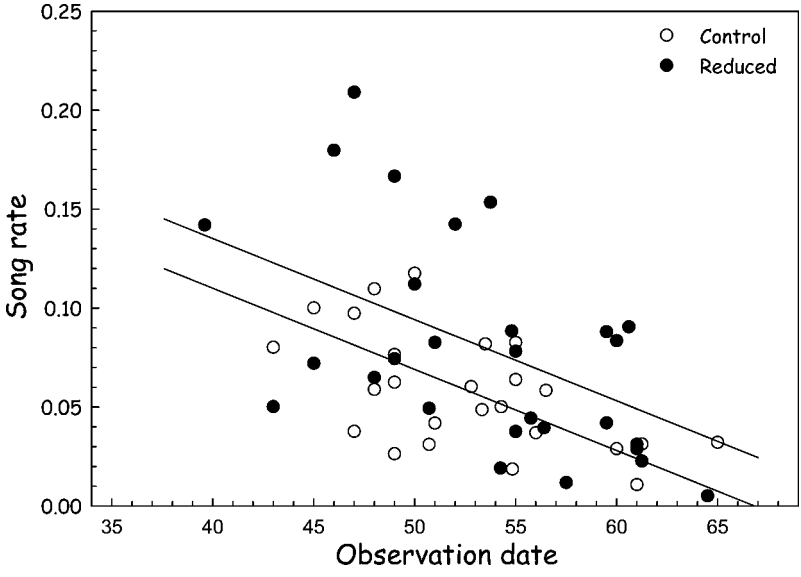

Figure 1. Song rate and observation date for males rearing control (○) or experimentally reduced (●) broods. Data are means per male. Date is expressed as 'April date' (1 May = day 31). Song rate was arcsine square root transformed.

$F_{36,115} = 3.37$, $P < 0.0001$), and comparable with an aspect of behaviour of the same males that can be measured with little error, namely feeding rate (also corrected for nestling age: $r = 0.50$, s.e. $= 0.08$, $F_{36,115} = 5.04$, $P < 0.0001$). Thus individual variation in song rate is measurable using our method.

Controlling for year, song rate declined with age and hatching date, and males rearing experimentally reduced broods had higher song rate. Coefficients of age and hatch date were virtually identical in this model (age: -0.0040, s.e. $= 0.0019$, $t = 2.24$, $P < 0.04$; hatch date: -0.0038, s.e. $= 0.0010$, $P < 0.001$), and therefore the model could be simplified by replacing these two variables with observation date (because observation date = hatch date + age at observation). The resulting model showed a significant seasonal decline in song rate, and a positive effect of brood size reduction (Fig. 1). Although the experimental effect was significant when years are pooled ($P < 0.006$), there was a significant interaction between year and experiment (Table 1), and the experimental effect was due to the first year only (Fig. 2). Original clutch size did not decrease the variance significantly when added to this model ($P = 0.8$).

High song rate of males with experimentally reduced broods can be interpreted as an indicator of the male's state, in the sense that males that have to work less hard appear to advertise their better state with higher song rate. If this were the case, a positive association between song rate and reproductive success is expected. Fledging success (the proportion of eggs remaining after manipulation that resulted in a fledged young) was not correlated with song rate when year and brood size manipulation were taken into account (logistic regression: $F_{1,49} = 0.7$, $P = 0.4$).

Table 1.
Song rate in relation to year, date and brood size manipulation. Song rate was arcsine square root transformed. Year was a dummy variable; 1990 = 0, 1991 = 1. Manipulation was also a dummy variable; control = 0, reduced = 1; n = 53 males, $R^2 = 0.46$.

Parameter	Coefficient (s.e.)	*t*	*P*
Constant	0.257 (0.044)	5.83	0.001
Year	−0.00214 (0.014)	0.15	0.9
Date	−0.0038 (0.00082)	3.62	0.001
Manipulation	0.050 (0.014)	3.62	0.001
Year * Manipulation	−0.041 (0.019)	2.15	0.04

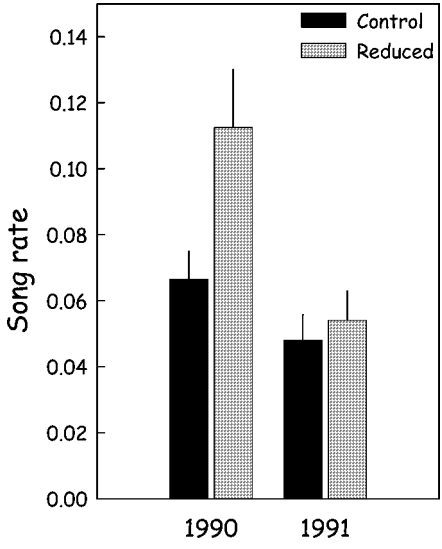

Figure 2. Average (s.e.) arcsine square root transformed song rate of males rearing control or reduced broods in the 2 study years. Song rate declines with age, and for comparability were all adjusted to an age of 10 days. For bars from left to right n = 14, 11, 11, and 17.

However, males with high song rate produced chicks with higher mass (Fig. 3), even when year and brood size manipulation are controlled for statistically (Table 2). This is important, because nestling mass is correlated with subsequent fledgling survival (Perrins, 1965). Hatch date, or interactions of song rate with year or manipulation, had no significant effect when added to this model (all $P > 0.3$).

The hypothesis that high song rate indicates that males have to work less hard can also be tested directly, by relating song rate to paternal effort. Two measures of effort are available in the present study: provisioning rate and daily energy expenditure (DEE). Provisioning rate increases with nestling age (Verhulst and Tinbergen, 1997), while song rate declines, and I adjusted both parameters to a nestling age of 10 days to account for the effect of nestling age. Overall, song rate declined weakly with increasing provisioning rate (controlling for year and hatch

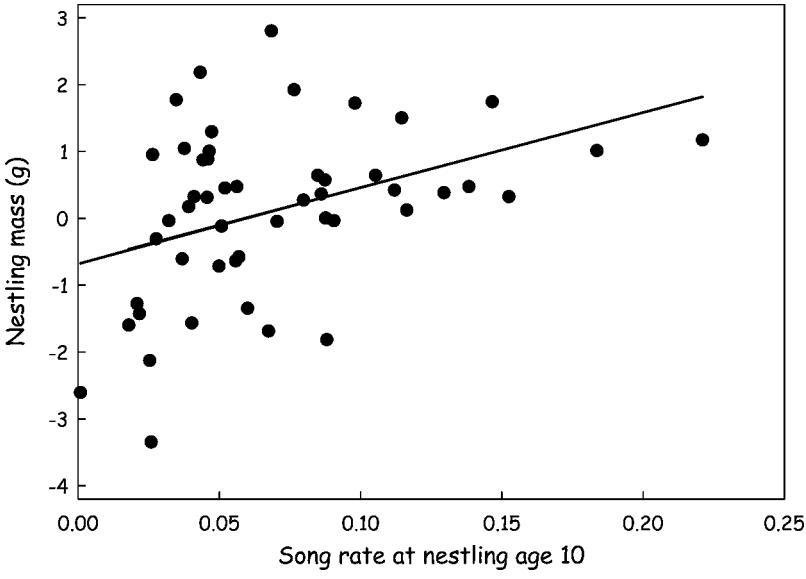

Figure 3. Nestling mass (mean per brood) and male song rate at nestling age 10. Nestling mass data are residuals calculated using the coefficients in Table 2.

Table 2.
Mean nestling mass (g) in relation to song rate, year, date and brood size manipulation. Song rate was adjusted value to nestling age 10 days. See Table 1 for explanation of other parameters. n = 50 broods, $R^2 = 0.51$.

Parameter	Coefficient (s.e.)	t	P
Constant	16.16 (0.44)	36.6	0.001
Year	−2.31 (0.53)	0.53	0.001
Manipulation	0.33 (0.53)	0.63	0.5
Year * Manipulation	1.83 (0.73)	2.53	<0.02
Song rate	11.24 (4.54)	2.47	<0.02

date, partial $r = -0.287$, n = 53, $P < 0.05$). This correlation is due to the brood size manipulation, because provisioning rate was not significantly correlated with song rate when brood size manipulation was also controlled for (partial $r = -0.15$, $P = 0.3$). Song rate was not correlated with paternal DEE at nestling age 12 (controlling for year and hatch date, partial $r = -0.03$, n = 25, n.s.).

A sexual conflict is likely to exist over parental investment in the first brood (Lessells, 1998), and song rate could play a role in resolving this conflict. This was investigated by testing the correlation between song rate (adjusted to nestling age 10), and the ratio between female and male parental effort. If song rate plays an important role in negotiating the conflict over parental effort, a positive correlation between song rate and relative female effort would arise. Pairs of which the females were already producing eggs for the second clutch at nestling age 12 (when we

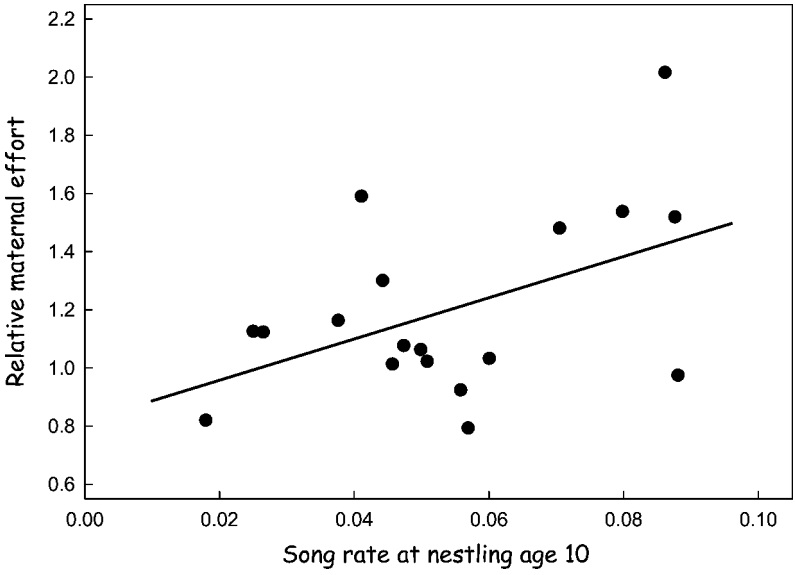

Figure 4. Song rate at nestling age 10 and relative female maternal effort (female daily energy expenditure / male daily energy expenditure) are significantly correlated ($r = 0.48$, n = 18, $P < 0.05$).

measured DEE) were omitted from this analysis (n = 3). Relative female DEE and provisioning rate were independent of clutch size manipulation (Verhulst and Tinbergen, 1997). Relative female provisioning rate (= female provisioning rate / male provisioning rate) was not correlated with song rate ($r = 0.17$, n = 50, $P = 0.2$), but female provisioning rate may not be a very informative measure of female parental investment, because it was not correlated with DEE (Verhulst and Tinbergen, 1997). Absolute female DEE was weakly positively correlated with song rate ($r = 0.28$, n = 18, $P = 0.3$), and there was a significant correlation between song rate and relative female DEE (Fig. 4). This correlation remained significant when year and hatch date were controlled for statistically (partial $r = 0.50$, n = 18, $P < 0.04$).

Does song rate have any effect on occurrence and timing of second clutches? By definition, second clutches are clutches produced after fledging young from the first brood, and therefore three broods that failed were omitted. In addition one female was omitted that changed mates between the first and second clutch (this is very rare). In both years, males with higher song rate were more likely to have a second clutch (Fig. 5). This is not surprising, because the two main factors explaining whether or not pairs start a second clutch are time of breeding and brood size (manipulation), and both correlated significantly with song rate (Fig. 1). Indeed, when these factors are controlled for in a logistic regression analysis, there is no additional effect of song rate on the probability that pairs produce a second clutch ($P > 0.8$). In pairs that start a second clutch, the males could be stimulated to sing more towards the end of the nestling phase, perhaps by the female's behaviour.

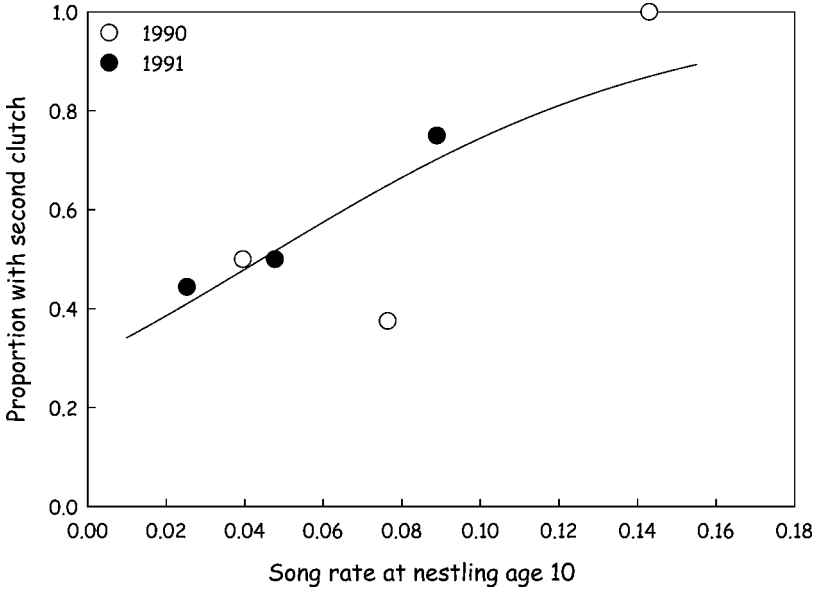

Figure 5. Song rate and the proportion of pairs starting a second clutch in the 2 study years. Song rate declines with nestling age, and for comparability arcsine square root transformed song rate values were adjusted to an age of 10 days. Symbols represent mean song rate and proportion with second clutch for the lowest, intermediate and highest 33% of the song rates in the two years. From left to right, sample size is eight for each data points in each year, except the first 1991 point for which n = 9. The proportion with a second clutch increased significantly with increasing song rate (logistic regression, $F_{1,48} = 4.6$, $P < 0.04$), and slope and level of this correlation were independent of year (both $P > 0.7$).

However, there was no indication that the decline in song rate with increasing age of the brood was different for pairs with or without a second clutch (interaction: $P = 0.8$).

If females are more likely to start a second clutch when their males sing more, they can also be expected to start the second clutch at an earlier date with increasing song rate, because from an optimality perspective these 'decisions' are closely related (Verhulst et al., 1997). Inter-clutch interval is defined as the number of days between the hatch date of the first clutch, and the date the first egg is laid of the second clutch. One family breeding on the edge of the village wood had a very long inter-clutch interval (>4 s.d. from the mean). We did not observe this family for some time after the first brood fledged, and suspected that they had produced a (failed) second clutch in a private garden that we had missed. This outlier was therefore omitted from the analysis. Controlling for year, inter-clutch interval significantly declined with increasing song rate (Fig. 6). This correlation remained significant when brood size manipulation (interacting with year) and hatch date were added to the model (partial $r = -0.47$, n = 28, $P < 0.02$). The number of eggs in the second clutch was not related to song rate ($P > 0.4$) when the inter-clutch interval was taken into account.

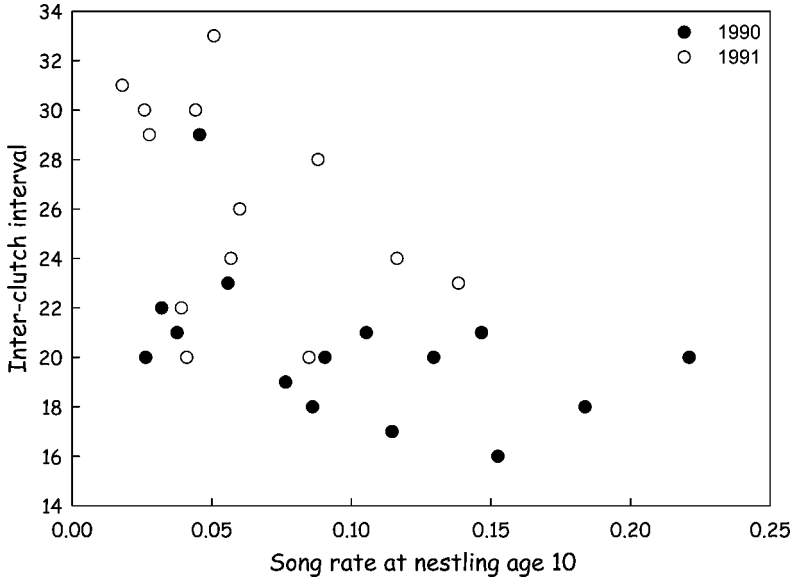

Figure 6. Song rate and inter-clutch interval (days). Controlling for year ($P < 0.01$), inter-clutch interval decreased with increasing song rate ($P = 0.03$, $R^2 = 0.50$, n = 28). The slope did not differ between years ($P = 0.4$). Song rate was arcsine square root transformed.

The relationship between song rate and fitness components was apparently restricted to the current breeding season, because song rate was not correlated with the male's probability of being recorded as breeding bird in the next year (capture rate of males that succeed in fledging young is almost 100%, and capture-recapture analysis which would account for variation in capture probability was therefore not carried out), or lay date or clutch size the next year (all $P > 0.8$).

DISCUSSION

The aim of this study was to explore the role of male song rate in resolving sexual conflicts over parental effort and multiple breeding. With increasing song rate, females worked harder for their brood relative to their partner (Fig. 4), and were more likely to produce a second clutch (Fig. 5). Furthermore, there was a negative correlation between song rate and the inter-clutch interval (Fig. 6). These results are in agreement with the hypothesis that male song rate is important in the negotiation of sexual conflicts over parental effort and the decision to start a second clutch. Signalling to resolve a sexual conflict may be common, given the success of other studies in demonstrating differential allocation in relation to the strength of a sexual signal (review, Sheldon, 2000), and the observation that signaling between pair members when reproduction is already in progress is common (Wachtmeister, 2001).

Song in great tits functions as a signal in both intra- and inter-sexual selection (Hinde, 1952; Krebs et al., 1981). During detailed observations at a few nest boxes males usually sang close to the nest box, and at a time that their mate was in or near the box (pers. observ.). Song during laying and incubation was also produced mainly near the nest box, when the female was in the box (Drent, 1983; Slagsvold et al., 1994). These observations suggest that song during reproduction is primarily directed at the mate, rather than at other conspecifics.

Date and brood size manipulation were strongly correlated with song rate (Fig. 1) and these factors also have a strong effect on timing and occurrence of second clutches (Verboven et al., 2001). Indeed, when hatch date and brood size manipulation were controlled for statistically, there was no additional effect of song rate on the occurrence of second clutches. However, this does not preclude the possibility that a high song rate persuades the female to start a second clutch; it may simply be the case that effects of time and brood size are mediated via song rate. This hypothesis is supported by the finding that the correlation between song rate and inter-clutch interval remained significant when date and brood size manipulation were controlled for statistically. Furthermore, second clutches were never followed by a third clutch, and song rate when rearing the second brood was almost zero when the chicks were 10 days old (21 observations on six broods). Nevertheless, the effects of song rate on second clutches emerging in this study are only correlations, and experiments are required to test whether they reflect causal relationships. Logan et al. (1990) performed such an experiment in mockingbirds *Mimus polyglottis* using a playback experiment, and concluded that there was a causal relationship between song rate and re-nesting interval. This supports the hypothesis that also in great tits there is a direct effect of song rate on multiple breeding.

I am aware of only two other experimental studies which investigated the effect of brood size on vocalisations. Brood size reduction from two to one squab in ring doves *Streptopelia risoria*, resulted in increased cooing rate of both parents (Ten Cate and Hilbers, 1991). Inter-clutch interval also decreased, and it was hypothesised that this was mediated through social stimulation of the female's endocrinological state (Ten Cate et al., 1993). Møller (1991) showed that manipulation of the number of young in the first brood of swallows *Hirundo rustica* had an effect on song rate during the fertile period of the second clutch. However, brood size manipulation has an effect on the inter-clutch interval in swallows too (Møller, 1993), and since song rate declines with date (Møller, 1991) which was not controlled for, it seems likely that the experimental effect was partly or fully via date rather than directly via brood size.

Previous studies of great tit song in a life history context have focussed on repertoire size more than on song rate (McGregor et al., 1981; Lambrechts and Dhondt, 1987), and found repertoire size to be an indicator of male quality and reproductive success. This paper is restricted to causes and consequences of variation in song rate, rather than repertoire size. However, it seems worthwhile to test, for example, whether large repertoire size can compensate for low song

rate in negotiation over the decision to start a second clutch, or whether brood size manipulation affects repertoire size. Data on song rate in this paper were collected during the day, but song rate is much higher at dawn, also during reproduction (Slagsvold et al., 1994) and could play a further role in the negotiation of sexual conflict.

What information is contained in high song rate? Experimental administration of testosterone to brood-rearing great tit males resulted in an increase in song rate (van Duyse et al., 2000), and this may indicate that in the present study males with a high song rate were signalling high testosterone levels. The experimental effect of brood size reduction on song rate was restricted to the first year of study (Fig. 2). This annual difference is in agreement with data on survival and growth of nestlings (Table 2), which indicate that in the second year even birds with experimentally reduced broods had to work at full capacity to raise their young, while feeding conditions in the first year were much better due to a better match between mean hatch date and the seasonal peak in caterpillar abundance (Verhulst, 1995; Verboven et al., 2001). In the first year paternal daily energy expenditure during brood rearing was also lower (Verhulst and Tinbergen, 1997), and song rate of control males tended to be higher (although not significantly so, $P = 0.1$), supporting this interpretation. This indicates that high song rates signal that males somehow had an easier life, a hypothesis that is further supported by the correlations of song rate with provisioning rate and nestling mass (Fig. 3). This interpretation is in agreement with experimental and correlational studies which generally demonstrated positive effects of food availability and nutritional state on song rate (reviews, Vehrencamp, 2000; Gil and Gahr, 2002).

The finding that song rate reflects male state suggests that song rate could act as a handicap signal, the honesty of which is maintained by the production costs (Zahavi, 1975; Vehrencamp, 2000). Although production costs may not be negligible during singing (Vehrencamp et al., 1989; Eberhardt, 1994; Oberweger and Goller, 2001), it seems unlikely that song rates observed in this study were sufficiently high (maximum approximately 4%) to have a discernible effect on daily energy expenditure. This suggestion is confirmed by the absence of a correlation between song rate and paternal daily energy expenditure. However, given that the male usually sings near the nest box, and that the female can probably perceive the male's distance with some accuracy, song rate may provide the female with information regarding the 'free time' the male has available. The fact that total time spent singing is small then becomes less important when trying to understand signalling cost. For example, singing for 50% of the times that the female is near the nest box may result in a small percentage of total time spent singing, but to perform at this rate the male may have to be present at the box for perhaps 50% of time (depending on feeding synchronisation), thereby seriously restricting time available for other activities. Thus, when song has to be combined with presence at a particular location to be effective as a signal, and since the moment that signalling

is effective cannot be fully predicted, the costs of a small amount of song may be amplified to a quantity that is sufficient to maintain the honesty of the signal.

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